E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(3): 365-368
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www.fisheriesjournal.com
Received: 17-03-2017
Accepted: 18-04-2017

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Effect of herbal feed supplement Phytozoi on Running Mortality Syndrome in white leg shrimp *Litopenaeus vannamei* (Boone, 1931) farming

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Abstract
The running mortality syndrome (RMS) is a major problem in shrimp *Litopenaeus vannamei* farming areas, in recent years farmers were facing production losses in shrimp due to this disease. The prevention and control of different diseases in shrimp farming aqua farmers using chemicals and antibiotics. These therapeutic chemical substances create environmental pollution and human health hazards. The herbal compounds are alternatives to chemical drugs for improving the immunity of the cultivable organisms in aquaculture. The commercial herbal feed supplement Phytozoi was applied @ 3g/Kg feed every after 40 days of culture (DOC) upto harvest in P2 and P3 experimental culture ponds compared with control ponds (P1 and P4). The results clearly indicate that the application of phytozoi herbal feed supplement in *Litopenaeus vannamei* in culture ponds showed increased survival percentages, feed intake, improved water quality, proper molting and also recovered hapatopancreases of the shrimp. The result clearly shows that there was significant ($P<0.01$) difference in survival percentages and total yields between the experimental ponds when compared to controls. The herbal feed supplement phytozoi may improve the resistance against the running mortality syndrome of shrimp and also offer economic benefits.

Keywords: Running mortality syndrome, *Litopenaeus vannamei*, Herbal feed supplement, Phytozoi

1. Introduction
Aquaculture is the fastest growing food production sector in worldwide, including Asian countries. It plays a vital role in offering better and quality nutrition, employment generation and rural development (Jayanthi *et al,* 2015) [3]. Shrimp culture in India has suffered problems from epidemics of various diseases, including viral and bacterial infections. Recently, the shrimp farmers were turned from the black tiger shrimp *Penaeus monodon* to *L. vannamei* farming in India due to frequent disease problems. After 2009, the white leg shrimp *Litopenaeus vannamei* is widely culture shrimp species at present in India. Several disease outbreaks have also been associated with vibriosis causing by bacteria such as vibrio species. In the past, most of the vibrio species has been considered to be secondary and opportunistic, and cause mortalities of animals under a deteriorated environment (Lightner *et al.,* 1984) [4]. Increasing in disease outbreaks have been reported to be associated with an increase in pathogenic vibrio populations of culture waters as well as multiple infections with vibrio species to cause mass mortality in many aquatic animals such as *Litopenaeus vannamei*.
Since 2011, a new undefined syndrome has a major problem in the shrimp culture in India (Masthan and Osman Ahmed, 2016) [8]. The farmed shrimp in the affected ponds show prolonged mortalities during the crop. There is no correlation to the water quality parameters and any other reported diseases earlier. This condition is called as "Running Mortality Syndrome" or RMS in shrimp. It is a problem currently faced by producers across Tamil Nadu and Andhra Pradesh. India's production of vannamei shrimp is likely to drop in 2015, as farmers face disease issues and consider a shift to producing black tiger (Neil Ramsden, 2015)[5]. The frequent disease problems along with environmental sustainability and persistence of pesticides, antiobiotics and other toxic chemicals in aquatic environment leads to accumulation of chemical residues in animal tissues posing a potential human health hazard. Currently scientists and farmers are using herbal products to improve immunity of cultivable organisms and reducing pathogenic bacteria load in culture pond environment. Several studies have been carried out to find the new compounds from plant sources at low cost and they are

the best to prevent the disease causing organisms in aquaculture (Sivasankar *et al.,* 2015) [7]. There are several medicinal plants used in shrimp aquaculture which includes *Aegle marmelos, Allium sativum, Azadirachta indica, Cassia fistula, Catharanthus roseus, Curcuma longa, Cynodon dactylon, Lantana camara, Morus alba, Ocimum americanum, Phyllanthus amarus, Phyllanthus emblica, Psidium guajava, Solanum nigrum, Tridax procumban and Tylophora indica* (Balasubramanian *et al*., 2007) [2]. The aim of the present study is to identify the effect of commercial herbal product "Phytozoi" on Running Mortality Syndrome (RMS), which was not conducted earlier in white leg shrimp *Litopenaeus vannamei* in semi- intensive culture ponds.

2. Materials and Methods

The present work was carried out in a private shrimp farm in Koppolu village near Ongole area (15° 30' 20.6028" N and 80° 2' 59.7084" E), Prakasam district, Andhra Pradesh, India. The study was conducted in four semi intensive shrimp culture ponds (P1, P2, P3 and P4). There was a separate reservoir pond about 0.8 ha maintained for treatment of raw water drawn from creek. The creek water salinity adjusted with the mixing of bore well water to maintain 10 ppt salinity in the culture ponds. Culture ponds adopted for this study were uniformly prepared, following usual practices like ploughing, liming etc. and were filled with filtered, chlorinated (30 ppm) and dechlorinated creek water up to 1.2 to 1.3 m depth. This was followed by manuring and fertilization for all ponds to grow the good plankton bloom in the pond water and enriched with probiotics with molasses for the growing of beneficial bacterial environment. After proper pond preparation *L. vannamei* seed post larvae (PL_{14}) were stocked @ 30/m^2 in P1, P2 and 35/m^2 in P3, P4 respectively obtained from private shrimp hatchery after proper screening of viral and bacterial contamination (Table:1). No water exchange was done during the culture period. However, required water filling was done from the reservoir at regular intervals to compensate water loss due to evaporation or soil seepage.

During pond culture water quality variables were maintained at optimal levels. After stocking post larvae were fed with CP shrimp feed (CP Aquaculture India Ltd., Chennai, India) for the entire culture duration in different feeding rations and schedule suggested in CP feed chart and adjusted according to proper check tray observation.

The herbal feed supplement Phytozoi (Biode Technologies, Bangalore) was mixed @ 3g/Kg feed after 50 days of culture (DOC) till harvest in P2 and P3 experimental culture ponds, the other culture ponds (P1 and P4) were also monitored with same pond management practices without application of herbal feed supplement, but usual treatment measures were followed with other supplements along with C vitamin for the control of mortalities in the culture ponds.

The mortalities, fecal matter, shrimp body colour, loss of appendages and gut fullness were observed daily at 6 am by visual monitoring the entire the pond from dykes and check tray observation. The water quality parameters like pH, Total Ammonia Nitrogen, Dissolved Oxygen, Total alkalinity, Nitrite-Nitrogen were determined in all the culture ponds following standard methods (APHA, 1995) [1]. The total yields, survivals and shrimp counts were observed using standard procedures at the harvest.

Data were statistically analyzed using one way analysis of variance (ANOVA) to find out any significant deference among the experimental groups and the comparison between treatments was done using Duncan's multiple range test (DMRT) at $P<0.05$ (Snedecor and Cochran, 1967) [10] (SPSS; 14.0 version).

Table 1: Experimental culture pond details

Culture ponds	Water spread area	Water depth (m)	Stocking Density (SD)
P1	0.5 ha	1.2 to 1.3	30 /m^2
P2	0.5ha	1.2 to 1.3	30 /m^2
P3	0.8 ha	1.2 to 1.3	35 /m^2
P4	0.8 ha	1.2 to 1.3	35 m^2

3. Results and Discussion

It is observed that the mortalities in *L. vannamei* culture ponds at 40th day of culture (DOC), about 8 animals were found dead in check tray, and increase in mortality was found heavily on day by day in all four culture ponds. Here the toxic elements are present other than bacteria, but the water quality parameters like pH, Total Ammonia Nitrogen, Dissolved Oxygen, Total alkalinity, Nitrite-Nitrogen were analyzed and found it in a normal range (Table:2). Through keen observation followed on the same day, it was assumed as epidemic disease and requires implementation of the health program in cultivable organisms. It is found that the moribund shrimps shows light pinkish to yellowish discoloration of the cephalothorax region (Fig: 3), reddening entire the body and blackened necrotic hepatopancreas were observed (Fig:1). The symptoms of the disease in the dead animals were noticed merely through a simple visual observation of the shrimp through daily check tray monitoring (Fig:7), where some signs can be evaluated. They were mainly, broken antennae (Fig:3); red uropods (Fig:2); white or yellow fecal matter in the gut (Fig:5); high mortalities observed during inter-molt stage (Fig:4),; dead shrimp settle at the bottom of the ponds were observed. The similar symptoms were also reported by Ravi Kumar and Sambasiva Rao (2014) [5], Srinivas *et al.* (2016) [9] and Mastan and Osman Ahmed (2016) [8].

Table 2: Group-wise Mean and Standard Error (± SE) of water quality parameters in control and experimental culture ponds.

Parameter	Control Ponds		Experimental Ponds	
	P1	P4	P2	P3
Temperature (^oC)	28.92±0.341^a	29.15±0.019^a	29.10±0.019^a	28.92±0.208^a
pH	7.97±0.224^a	8.15±0.127^b	8.19±0.180^c	8.25±0.164^d
Dissolved Oxygen (ppm)	4.15±0.224^a	5.13±0.180^b	5.19±0.127^b	5.30±0.164^c
Total Alkalinity (ppm)	185.70±0.107^a	165.11±0.112^b	152.48±0.126^c	162.29±0.171^d
Total Ammonia – Nitrogen (ppm)	0.95±0.224^c	0.44±0.127^a	0.56±0.112^b	0.48±0.180^a
Nitrite – Nitrogen (ppm)	0.0065±0.224^b	0.0031±0.107^a	0.0030±0.127^a	0.0030±0.180^a

[Means having the same superscript in each row do not differ significantly ($P<0.05$) amongst themselves; DMRT (Duncan's multiple range test)]

It is also assumed that the disease might have been caused due to the variance in degrees of high temperature. The treatment for this disease was done through several attempts with different commercially available feed supplements along with "C" vitamin with different dosages (3g/ Kg; 5g/ Kg feed) which was suspected to be caused by the Vibrio disease. The results envisage that for 5g/kg dosage improved less than 5% of the shrimp health in 24 hours after the treatment and there was a recovery of less than 10% only. The same feed supplements were also used in two experimental ponds (P2 and P3), but those were failed to control prolonged mortalities caused by the unknown etiology.

It is evident from the results that by using the herbal feed supplement Phytozoi in two experimental culture ponds (P2 and P3) improved survivals, activeness and feed intake in the cultivable organisms. The immunostimulant properties of Phytozoi played a positive role in enhancing the beneficial effect on health of the shrimps in culture ponds. The observations indicate that the feed consumption has been increased in both the treatment ponds, proper molting behaviour and the hepatopancreas of shrimp turned pale color to olive green color was observed in treatment ponds and controlled the daily mortality in culture ponds. The result also clearly shows that there were significant ($P<0.01$) difference in survival percentages and total yields between the experimental ponds (P2 and P3) and controls (P1 and P4). Better survival is a crucial factor in achieving good production and good counts in shrimp culture.

4. Conclusion

Generally, use of herbal supplements and extracts does not lead to drug resistance. Herbal products are known to play an important role in disease control because they contain active ingredients with antioxidants, antimicrobial property, anti-stress, growth promotion, appetite stimulation, disease resistance and immunostimulation. The herbal feed supplement Phytozoi may be involved to prevent the entry of disease causative organisms into the pond system, minimize the effects of disease by reducing stress to the shrimp, which may boost their disease resistant and stimulate molting process regularly. The use of herbal supplements instead of antibiotics and chemicals in shrimp farming is an alternative to reduce the problems related to tissue biomagnifications that in turn leads to rejection of the total consignment during the export.

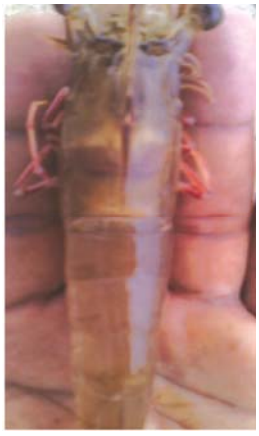

Fig 1: shrimp *L. vannamei* reddening entire body

Fig 2: Red coloured uropods

Fig 3: light pinkish to yellowish discoloration of the cephalothorax region and reddening entire body

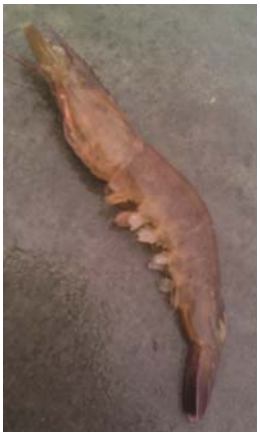

Fig 4: Mortality during inter-molt stage

Fig 5: Yellow fecal matter

Fig 6: Normal Shrimp

Fig 7: Dead shrimps in check tray

5. References

1. APHA, Standard methods for the examination of water and waste water edited by Stand, M.A. and Fauson, H. (American Public Health Association, Washington DC, USA), 1995.
2. Balasubramanian G, Sarathi M, Rajesh Kumar S, Sahul Hameed AS. Screening the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp. Aquaculture. 2007; 263:15-19.
3. Jayanthi L, Saravana Bhavan P, Srinivasan V, Muralisankar T, Manickam N. Probiotics product (Lacto Bacil Plus) on improvement of survival, growth, digestive enzymes activity, nutritional status and gut microflora of the prawn *Macrobrachium rosenbergii*. International Journal of Current Research 2015; 7(01) ISSN: 0975-833X; pp.11440-11453.
4. Lightner DV, Redman RM, Danald DA, Williams RR, Perez LA. Major diseases encountered in controlled environment culture of penaeid shrimp at Puerto Penasco, Sonora, Mexico. Sindermann CJ, ed. Proceedings of the ninth and tenth U.S.-Japan Meetings on Aquaculture; Lewes; Delaware, U.S.A. Seattle, Washington: National Marine Fisheries Service, NOAA; 25-33. (NOAA Technical report NMFS, 16), 1984.
5. Neil Ramsden. New disease has Indian shrimp farmers mulling return to black tiger. Undercurrent News, 2015. https://www.undercurrentnews.com.
6. Ravi Kumar A, Sambasiva Rao KRS. Running Mortality Syndrome-Progressive mortality in shrimp farming ponds, Fishing Chimes. 2014; 34(5-6):13-15.
7. Sivasankar P, Anix Vivek Santhiya A, Kanaga V. A review on plants and herbal extracts against viral diseases in aquaculture. Journal of Medicinal Plants Studies 2015; 3(2):75-79.
8. Mastan SA, Md. Osman Ahmed. Running mortality syndrome (RMS) in farm- reared shrimp, *Litopenaeus vannamei* culture systems in Andhra Pradesh, India. Indo American Journal of Pharmaceutical Research, 2016. ISSN No: 2231-6876.
9. Srinivas D, Venkatryalu Ch, Laxmappa B. Identifying diseases affecting farmed *Litopenaeus vannamei* in different areas of Nellore district in Andhra Pradesh, India, International Journal of Fisheries and Aquatic studies. 2016; 4(2):447-451.
10. Snedecor GW, Cochran G. Statistical Methods. Oxford and IBH Publishing, New Delhi, India, 1967, 593.